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Response of a hypernodulating soybean mutant to increased photosynthate supply

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Abstract

Growth chamber studies were conducted to determine if increased photoassimilate supply, through light enhancement and CO_2 enrichment, could reverse the deleterious plant growth and enhance nodule function traits of NOD1-3, a hypernodulating mutant of Williams. Both light enhancement and CO_2 enrichment increased nodule number, acetylene reduction activity plant^{-1} (but not specific activity) and dry matter accumulation in all tissues in both genotypes. Total biomass and specific nitrogenase activity were always less in the mutant than in Williams 82, indicating that the inferiority of the mutant may not be reversed by enhanced photoassimilate supply. Under all growth conditions, the mutant allocated relatively more photosynthate to nodules and less photosynthate to roots, compared to the control. Despite this, the decreased growth of the mutant relative to the control was not solely attributable to excessive nodulation of the mutant, since decreased growth was observed even on uninoculated plants. It is suggested that light enhancement and CO_2 enrichment may have stimulated nodulation through increased photosynthate supply, independent of the nodulation autoregulatory signal. © 1997 Elsevier Science Ireland Ltd.

Keywords: Glycine max; *Bradyrhizobium japonicum*; Hypernodulation; CO_2 enrichment; Light enhancement

1. Introduction

It is well established that nodulation and N_2 fixation are markedly repressed when legumes are grown on media containing combined nitrogen [1,2]. Since this is generally the case under agricultural situations, efforts have been made to select legumes for which nodulation and N_2 fixation are not repressed by the presence of combined nitro-

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[2] Trade and manufacturers names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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gen. Following chemical mutagenesis, partially nitrate-tolerant hyper- or super-nodulating soybean mutants have been selected [3–5]. However, hyper- or super-nodulating mutants have decreased growth [4,6,7] and a low specific nitrogenase activity relative to respective wild-type parents [8–10]. Although decreased growth of the mutants was observed in absence of inoculation [4,6,10], studies with Bragg and its supernodulating nitrate-tolerant symbiotic (*nts*) mutants indicated that the excessive nodulation accentuates the problem [6,11]. Gresshoff et al. [12] suggested that the *nts* mutants of Bragg resulted from a single mutation with a pleiotropic effect, implying that supernodulation may be the cause of the decreased growth of the mutants.

Soybean plants regulate nodule number through an internal autoregulatory system. It was suggested that the autoregulatory mechanism of nodulation is induced by the sub-epidermal cell division stage of nodule ontogeny and results in suppression of development of subsequent subepidermal cells into emergent nodules [13,14]. Delves et al. [15], Cho and Harper [16] and Hamaguchi et al. [17] used grafting experiments to show that the nodulation phenotypes (normal nodulation or super- or hyper-nodulation) were controlled by the shoot, regardless of the root genotype. In an effort to isolate the nodulation autoregulatory compound(s), experiments have been conducted to further localize their sources in the shoot. Continuous removal of apical and lateral meristem apices demonstrated that autoregulation of nodulation was not significantly altered by absence of the shoot apex in either supernodulating or normally nodulating shoots [18]. It was suggested that the nodulation autoregulatory signal may reside in the leaf rather than in the shoot apex. Recently, by successive removal of various shoot parts (cotyledons, primary leaves, and shoot apices) and by inducing leaves and shoot cuttings to root and nodulate, Francisco and Harper [19] definitively showed that the autoregulatory signal is derived from the leaf.

Mellor and Collinge [20] hypothesized that the nodulation autoregulatory compounds may involve Nod factor-degrading enzymes, such as chitinases, or *Nod* gene-down-regulating compounds, such as riboflavin, acetosyringone, ubiquinone, etc. Data from studies of nodulation mutants, however, indicate that *Nod* gene-inducing substances [16] or levels of induction of *Nod* genes [21] may not be involved.

The fact that shoots from supernodulating or hypernodulating genotypes supply less photosynthate for root growth and nodulation than normally nodulating genotypes, yet are able to induce supernodulation or hypernodulation and that shoots from normally nodulating genotypes are able to suppress supernodulation or hypernodulation, indicates that a signal other than photosynthate supply is involved in autoregulation of nodulation. However, availability of photosynthate has also been shown to be involved in the control of nodule number [7,19,22]. Francisco and Harper [7] showed that delayed inoculation increased nodule number, presumably due to an increased number of infection sites. Therefore, any factor that increases root growth may result in an increased nodule number. Approach grafting of a second shoot to any root of the same stock resulted in an increase in nodule number in all nodulation phenotypes, presumably through increased photosynthate supply to the root stock [19]. Barbera and Harper [22] found that more nodules formed on mung bean roots when soybean was the shoot than when mung bean was the shoot. They attributed this to more photosynthate availability when soybean was the shoot, as soybean shoot growth was more than five-fold greater than mung bean shoot growth. Identification of key environmental factors impacting autoregulation of nodulation may help identify the signal. Other than response to N supply, such studies are lacking. Moreover, in experiments carried out to localize the nodulation autoregulatory signal and to investigate the effect of environmental factors on the levels of the signal, control of nodule number by availability of photosynthates should be distinguished from the control by the nodulation autoregulatory signal(s) and photosynthetic light effects should be distinguished from non-photosynthetic effects.

Experiments were conducted to investigate (i) whether enhanced photoassimilate supply could compensate for the deleterious growth traits of

the hypernodulated mutant and its decreased specific nitrogenase activity; (ii) whether the decreased growth of the mutant is attributable to photosynthate drain by the excessive nodulation; and (iii) whether autoregulation of nodulation was altered by availability of photosynthates. Growth, photosynthate partitioning, nodulation and nodule function of NOD1-3 (hypernodulating mutant) and Williams 82 (normally nodulating line) were compared under various light and CO_2 combinations, under limited versus extended N supplementation, under half-strength versus full-strength nutrient solution and under continuous light versus light/dark cycles. Growth of the two genotypes was also contrasted under inoculated versus uninoculated conditions.

2. Materials and methods

2.1. Plant material and culture conditions

A normally nodulating soybean (*Glycine max* (L.) Merr. cv. Williams 82) and a hypernodulating mutant (NOD1-3) derived from cv. Williams were used. Williams 82 is an isoline of Williams which contains single gene resistance to *Phytophthora* and is currently being used as a normally nodulating control for the hypernodulated line (NOD1-3). Seeds were surface-sterilized by soaking in 70% ethanol for 1 min, then in 3% NaClO for 3 min. After a thorough rinsing in sterile deionized water, seeds were germinated in sand trays. Seven-day-old seedlings were suspended through lids of 2-L polyethylene containers containing a modified full-strength Hoagland nutrient solution as described by Schweitzer and Harper [23]. The standard growth solution contained 1 mM urea the first week (limited N supplementation) or the first 2 weeks (extended N supplementation) of growth and was N free thereafter. Exceptions are noted with treatment descriptions below. Previous studies had shown that under high light or enriched CO_2, growth of soybean plants that relied on N_2 fixation as the sole source of N was N limited [24] and that autoregulation of nodulation was related to N availability [3–5]. Thus, to optimize growth and photosynthate availability without masking the potential effect of increased photosynthate availability on autoregulation of nodule number by the effect of N, growth and autoregulation of nodulation response to high light and/or enriched CO_2 in the two genotypes were determined under extended versus limited N supplementation as urea. All hyper- or super-nodulating mutants characterized so far have a smaller root system relative to their parents [3–5]. To determine whether this decreased root growth is responsible for the decreased overall growth of the mutant due to inefficient nutrient acquisition, growth of the two genotypes was also compared under a half-strength versus a full-strength nutrient solution. The full-strength nutrient solution was also used to potentially enhance growth under high light and/or enriched CO_2. Since phosphorous toxicity in soybean is know to occur at low levels of nitrate [25], we routinely use very low levels of phosphorus (0.05 mM) in the absence of nitrate in the nutrient solution. The resulting low buffering capacity was overcome by including ion exchange resin columns in the nutrient solutions [26]. Columns were connected to an air supply to recirculate the nutrient solution to maintain the solution pH above 5.5 and to provide continuous aeration. The 7-day-old plants were inoculated with a solution of *Bradyrhizobium japonicum* strain USDA 110, at about 10^{10} bacteria per 2-L container. Plants were grown in controlled environmental chambers, that provided a 14 h photoperiod, a 28/20°C day/night temperature regime and a relative humidity of about 55%.

2.2. Light and CO_2 treatments

Plants were grown either in chambers that provided a photosynthetically active radiation (PAR) at the top of the canopy at harvest of about 300 μmol m^{-2} s^{-1} (low light) or in chambers that provided a PAR of about 800 μmol m^{-2} s^{-1} (high light). The desired PAR was attained by combining 1500 mAmp cool-white fluorescent, 100 W incandescent and 130 W low pressure sodium lamps, as measured with a Li-Cor Li-185A photometer (Lambda Institute, Lincoln, NE). The CO_2 level was either ambient (about 400 μmol mol^{-1}) or enriched (about 1000 μmol

mol^{-1}). The 1000 μmol mol^{-1} CO_2 concentration was chosen because it approximately represents the CO_2-saturated leaf photosynthesis in non-stressed soybean [27]. The CO_2 enrichment was obtained by introducing tank CO_2 into a sealed growth chamber containing the plants. The CO_2 flow rate from the tank was adjusted to maintain the desired CO_2 level as monitored by a Li-Cor 6200 system equipped with a Li-6250 infrared gas analyzer (Li-Cor, Lincoln, NE). In another experiment, plants were grown continuously on 15 mM nitrate with either continuous low light or low light/dark cycles. The 15 mM nitrate/continuous light combination was included to evaluate whether nodulation response to nitrate was altered in response to enhanced photosynthate input, relative to the known inhibitory effect of this level of nitrate under diurnal growth conditions.

2.3. Nitrogenase activity (acetylene reduction activity, ARA) and tissue dry matter

A previous study [24] showed that the onset of nitrogenase inhibition by nitrate was within 24 h of nitrate treatment. Therefore, sampling for ARA determination was done 24 h following nitrate treatment. The detached root method was used. This method has been criticized because of a possible acetylene-induced decline in nitrogenase activity, which may differ between stressed and control treatments; and because excision of the shoot may result in an underestimation of absolute nitrogenase activity due to disturbance, carbohydrate limitation, etc. However, this potential problem of using ARA on excised roots does not appear to be universal [2,28] and it was not observed under our assay conditions: there was no acetylene induced decline in nitrogenase activity; ethylene formation was linear over the 30 min assay period. This lack of the acetylene-induced decline in nitrogenase activity was not due to the fact that nitrogenase was already inhibited by other factors because this was observed on both treated and control plants, on both excised roots and intact undisturbed plants in a closed acetylene feeding system. We were interested in relative differences in nitrogenase activity between treatments and Vessey [29] has shown that over a wide range of nitrogenase activities, ARA determined on excised roots in a closed system predicted the same difference in ARA between treatments as did the open system on intact undisturbed plants. Therefore, the method appeared to be valid for our purpose. Assay for ARA was carried out as described by Bacanamwo and Harper [24] and ARA (μmol ethylene $plant^{-1}$ h^{-1}) was used as an estimate of nitrogenase activity. After ARA determination, nodules were detached from roots and counted. Then nodules, roots and shoots were dried at 80°C in an oven to a constant weight and dry matter of the various fractions was determined. Unless otherwise specified, sampling for ARA and tissue dry matter determination was made 21 days after transplanting.

3. Results

3.1. Response of growth and nodule number to increased photosynthate supply

When N deficiency was alleviated by extended N (urea) supplementation, both CO_2 enrichment (experiment I) and light enhancement (experiment II) increased dry matter accumulation in nodules, roots and shoots of both Williams 82 and the NOD1-3 hypernodulating mutant (Table 1). Dry matter increase following CO_2 enrichment was more pronounced in NOD1-3 than in Williams 82 (experiment I). Increases in nodule, root and shoot dry matter were 22, 60 and 40%, respectively, in Williams 82; and 47, 67 and 49%, respectively, in NOD1-3. The increase in dry matter following light enhancement was more pronounced in Williams 82 than in the NOD1-3 mutant. Nodule, root and shoot dry matter increased by 54, 42 and 48%, respectively, in Williams 82; and by 22, 36 and 30%, respectively, in NOD1-3 in response to light enhancement (experiment II). The high light × enriched CO_2 combination gave the highest dry matter increase in both genotypes (experiment II). Relative to Williams 82, increased nodule number in the mutant was more favored by CO_2 enrichment than by light enhancement. Increases in nodule number

Table 1
Effect of increased photosynthate supply with extended urea supplementation on soybean growth and nodulation

Cultivar	Light × CO_2 combinations	Nodule number (plant^{-1})	Dry matter (mg plant^{-1})		
			Nodule	Root	Shoot
Experiment I					
W82	L × A	95 ± 5	73 ± 6	388 ± 14	1260 ± 30
W82	L × E	157 ± 7	89 ± 5	620 ± 28	1760 ± 59
NOD1-3	L × A	320 ± 13	119 ± 5	153 ± 6	706 ± 18
NOD1-3	L × E	507 ± 34	175 ± 10	256 ± 16	1050 ± 35
Experiment II					
W82	L × A	108 ± 9	56 ± 6	390 ± 21	1120 ± 50
W82	H × A	155 ± 12	86 ± 2	553 ± 8	1660 ± 32
W82	H × E	185 ± 20	114 ± 13	698 ± 33	1890 ± 56
NOD1-3	L × A	571 ± 46	140 ± 9	180 ± 8	741 ± 19
NOD1-3	H × A	684 ± 42	171 ± 5	244 ± 8	967 ± 28
NOD1-3	H × E	582 ± 40	201 ± 5	312 ± 4	1070 ± 22

L, low light; H, high light; A, ambient CO_2; E, enriched CO_2. Williams 82 (W82) and NOD1-3 (hypernodulating mutant) cvs. were germinated in sand trays and 7-day-old seedlings were inoculated and transplanted to a half-strength nutrient solution. Transplanted plants were grown in growth chambers at low and high light in combination with ambient and enriched CO_2 in two separate experiments. Low and high light levels were 300 and 800 μmol m^{-2} s^{-1}, respectively, while ambient and enriched CO_2 levels were 400 and 1000 μmol mol^{-1}, respectively. The nutrient solution involving extended urea supplementation treatment contained 1 mM urea until 1 week before sampling at 21 days after transplanting. Values are means ± S.E. ($n = 6$ for experiment I and $n = 11$ for experiment II).

in the mutant were 58 and 20%, respectively, for CO_2 enrichment (experiment I) and light enhancement (experiment II), while increases in Williams 82 were 65 and 44%, respectively.

With the half-strength nutrient solution with urea-supplementation for 1 week, light enhancement at ambient CO_2 did not increase shoot and total dry matter in either genotype, while dry matter was increased by the CO_2 enrichment at low light in both genotypes (Table 2). The high light × enriched CO_2 combination increased total dry matter in Williams 82, but not in the NOD1-3 mutant (Table 2). At full-strength nutrient solution however, light increased dry matter accumulation in all tissues of both genotypes (Table 3).

Under all these growth conditions, growth of the NOD1-3 mutant was always less than that of Williams 82 control. To further determine whether or not the decreased growth of the mutant resulted from carbohydrate drain by the excessive nodulation, growth of the two genotypes was compared (i) at transplanting (7 days after germination, before inoculation); (ii) before significant nodule development (10 days after inoculation, when nodules had just emerged but still had a negligible weight) versus when uninoculated; and (iii) when grown on 15 mM nitrate (i.e. under conditions where their nodule number and nodule dry matter were negligible). As early as 7 days after germination and before inoculation, shoot growth of NOD1-3 (87 ± 4 mg plant^{-1}) was less than for Williams 82 (122 ± 6 mg plant^{-1}), while root growth was still similar (51 and 50 mg plant^{-1}, respectively). Seedling growth was again evaluated 10 days later, following transplanting and either inoculated or uninoculated (Table 4). Both root and shoot growth of NOD1-3 was significantly less than for Williams 82 under all light × CO_2 treatment combination, whether inoculated or not. Inoculation resulted in similar or less root and shoot dry matter for Williams 82, relative to uninoculated control, while no difference existed in root or shoot dry matter of NOD1-3 with and without inoculation (Table 4). When plants were grown on a full-strength nutrient solution with 15 mM nitrate, growth under continuous low light dramatically increased dry matter accumulation in various tissues relative to growth under a day/night light cycle (Table 5). Growth of the mutant was, however, still inferior

Table 2
Effect of light enhancement and CO_2 enrichment with limited urea supplementation on soybean growth and nodulation

Light × CO_2 combinations	Nodule number (plant^{-1})	Dry matter (mg plant^{-1})			
		Nodule	Root	Shoot	Total
Cultivar W82					
L × A	218^{d}	132^{c}	628^{b}	1580^{c}	2340^{c}
H × A	243^{d}	132^{c}	644^{b}	1440^{c}	2220^{c}
L × E	162^{d}	128^{c}	667^{b}	2190^{b}	2980^{b}
H × E	234^{d}	180^{b}	970^{a}	2510^{a}	3660^{a}
Cultivar NOD1-3					
L × A	862^{c}	214^{b}	292^{d}	988^{d}	1490^{e}
H × A	1030^{b}	285^{a}	425^{c}	1060^{d}	1770^{d,e}
L × E	1190^{a}	258^{a}	302^{d}	1390^{c}	1950^{c,d}
H × E	950^{b,c}	277^{a}	388^{c,d}	1370^{c}	2080^{c,d}

L, low light; H, high light; A, ambient CO_2; E, enriched CO_2. Other plant growth and treatment conditions as in Table 1 legend, except that the nutrient solution contained 1 mM urea only the first week of growth and was N-free thereafter. Within columns, means with the same letter are not significantly different at the 5% probability level using LSD.

to the control even though nodulation was not a factor.

3.2. Nodulation and its autoregulation response to increased photosynthate supply

When N deficiency was alleviated by extended N supplementation, both CO_2 enrichment (experiment I) and light enhancement (experiment II) increased nodule number in both genotypes (Table 1). However, excess photosynthate supply (high light under a full-strength nutrient solution (Table 3), or continuous light (Table 5)) tended to decrease nodule number especially in the NOD1-3 mutant. Under limited urea supplementation and half-strength nutrient solution, either light enhancement or CO_2 enrichment increased nodule number in the hypernodulating mutant NOD1-3, but nodule number in Williams 82 was essentially unaffected (Table 2). Under limited urea supplementation and full-strength nutrient solution, light enhancement decreased nodule number in the mutant, but nodule number in Williams 82 was little affected (Table 3).

3.3. Response of photosynthate partitioning and nitrogenase activity to increased photosynthate supply

Both increased light intensity and CO_2 enrichment increased total nitrogenase activity in both genotypes, but specific nitrogenase activity was little affected (Table 6). Specific nitrogenase activity in the NOD1-3 mutant remained less than in the Williams 82 control at all light and CO_2 levels. Distribution of photosynthates between shoot and root, as estimated by the root:shoot ratio, indicated that allocation to roots was also increased by light enhancement and CO_2 enrichment in both genotypes (Table 6). However, allocation of photosynthates to nodules, as estimated by the total plant mass:nodule mass ratio, was little affected by the increased photosynthate supply due to CO_2 enrichment or light enhancement. Under all growth conditions, the mutant allocated relatively less photosynthates to roots and relatively more photosynthates to nodules than the control.

4. Discussion

4.1. Growth and nodule activity in response to increased photosynthate supply

The increased plant growth noted with both light enhancement and CO_2 enrichment in both genotypes (Tables 1–5) is consistent with previous work in soybean [11,30–32]. Nitrogenase activity (C_2H_2 reduction) was also increased by the light

Table 3
Effect of light levels at ambient CO_2 with limited urea supplementation on soybean growth and nodulation

Cultivar	Light treatment	Nodule no. (plant^{-1})	Dry matter (mg plant^{-1})		
			Nodule	Root	Shoot
W82	Low	303^{c}	120^{c}	510^{b}	2020^{b}
W82	High	247^{c}	170^{b}	1170^{a}	2940^{a}
NOD1-3	Low	1109^{a}	190^{b}	210^{d}	1010^{d}
NOD1-3	High	793^{b}	300^{a}	340^{c}	1430^{c}

Other plant growth and treatment conditions are as in the legend of Table 1, except that the nutrient solution was full strength and contained 1 mM urea only during the first week of growth and was N-free thereafter. Within columns, means with the same letter are not significantly different at the 5% probability level using LSD.

enhancement and CO_2 enrichment although specific nitrogenase activity was essentially unaffected (Table 6). Failure to increase specific nitrogenase activity in normally nodulating soybean through light enhancement [32] or CO_2 enrichment [30] has been reported and the hypernodulating mutant does not appear to be an exception. Total biomass and specific ARA of NOD1-3 were always less than that of the Williams 82 control, even under enriched CO_2 where the mutant was more favored than the control. This indicates that the inferiority of the mutant will not be reversed by enhancement of photoassimilate supply. This also may indicate that although the mutant allocates relatively more photosynthates to nodules than does the wild type (Table 6), the decreased growth of the mutant may not be solely attributed to carbohydrate drain by the excessive nodulation. This photosynthate allocation pattern was not altered by increased photosynthate availability. This idea is substantiated by the fact that the decreased growth of the mutant was also observed in the absence of inoculation and that inoculation did not decrease shoot or root growth in the mutant (Table 4). Also when plants were grown continuously on 15 mM NO_3^-, nodule number and nodule dry matter in both Williams 82 and NOD1-3 was negligible, yet shoot and root dry matter were still decreased in NOD1-3 relative to Williams 82 (Table 5). Gresshoff et al. [12] also reported that inoculation caused a temporary decrease in shoot growth in Bragg but not in its supernodulating mutant *nts* 382. Decreased growth of the NOD1-3 mutant relative to Williams 82 when uninoculated and grown on combined N was also found by Gremaud and Harper [4]. Similarly, supernodulated mutants of Bragg were found to have decreased root growth in absence of inoculation [6].

Growth of Williams 82 was favored by light enhancement more than that of NOD1-3, while CO_2 enrichment favored growth of the NOD1-3 mutant over that of the Williams 82 control. Since light enhancement has been shown to increase respiration while CO_2 enrichment decreases it [33], we speculate that growth of the mutant may be more limited by respiration or photorespiration than is Williams 82. This is consistent with the findings of Day et al. [6] where the supernodulating mutant *nts* 382 exhibited higher respiratory rates than its parent cv. Bragg. The increased root:shoot ratio in both genotypes under increased light and CO_2 supply is in agreement with the general observation [34] that when a resource that is acquired by the shoot is in excess, more photosynthates are allocated to the roots and vice versa. Under all growth conditions, root:shoot ratio was always less in NOD1-3 than in Williams 82. However, there was no indication that the decreased root system in the mutant was responsible for the decreased overall growth of the mutant, as growth of the mutant relative to the control was not improved by use of a full-strength nutrient solution (Table 3), relative to use of a half-strength nutrient solution (Table 1).

Table 4
Growth response of inoculated and uninoculated soybean plants to increased photosynthate supply with extended urea supplementation

Light × CO_2 combinations	Dry matter (mg plant^{-1})			
	Root + nodule		Shoot	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Cultivar W82				
L × A	163 ± 5	134 ± 6	341 ± 21	305 ± 15
H × A	195 ± 14	200 ± 7	430 ± 18	352 ± 19
H × E	300 ± 5	288 ± 9	584 ± 21	535 ± 21
Cultivar NOD1-3				
L × A	118 ± 4	122 ± 13	221 ± 8	238 ± 10
H × A	149 ± 11	163 ± 3	286 ± 24	258 ± 18
H × E	220 ± 20	228 ± 9	418 ± 30	410 ± 15

L, low light; H, high light; A, ambient CO_2; E, enriched CO_2. Williams 82 (W82) and NOD1-3 (hypernodulating mutant) were germinated in sand trays and 7-day-old seedlings were transplanted to a half-strength nutrient solution, either inoculated or uninoculated. Transplanted plants were grown in growth chambers at low light, ambient CO_2; high light, ambient CO_2; or at high light, enriched CO_2. Low and high light levels were 300 and 800 μmol m^{-2} s^{-1}, respectively, while ambient and enriched CO_2 were 400 and 1000 μmol mol^{-1}, respectively. Plants were sampled 10 days after transplant. The nutrient solution contained 1 mM urea during the 10-day growth period. Values are means ± S.E. ($n = 6$).

4.2. Nodulation and autoregulation response to increased photosynthate supply

Under limited N supplementation, increases in light and CO_2 levels at half-strength nutrient solution increased nodule number in the hypernodulating mutant while nodule number in the normal nodulating control was little affected (Table 2). Increasing light level at full-strength nutrient solution decreased nodule number in the hypernodulated mutant and again, nodule number in the normal nodulating control remained essentially unaffected (Table 3). Nodule number in the NOD1-3 hypernodulating mutant was more responsive to change in environmental factors than was the Williams 82 control. This may reflect the altered autoregulation of nodulation in the mutant. This same conclusion was reached in the Bragg background by Hansen et al. [11]. They found that changes in light intensity altered nodule number more in the supernodulating mutants than in the Bragg parent. When N deficiency was

Table 5
Effect of light pattern on soybean growth and nodulation

Light	Cultivar	Nodule no. (plant^{-1})	Dry matter (mg plant^{-1})		Root/shoot ratio
			Root	Shoot	
Cycle	W82	0.67^{c}	650^{c}	2790^{c}	0.22^{a}
Continuous	W82	0.00^{c}	1370^{a}	5820^{a}	0.23^{a}
Cycle	NOD1-3	11.33^{a}	500^{d}	2300^{d}	0.22^{a}
Continuous	NOD1-3	3.33^{b}	960^{b}	4110^{b}	0.23^{a}

Williams 82 (W82) and NOD 1-3 (hypernodulating mutant) were grown on a full-strength nutrient solution containing 15 mM nitrate for 21 days. Plants were grown in growth chambers at either continuous low-light (300 μmol m^{-2} s^{-1}) or a diurnal light/dark cycle. Within columns, means with the same letter are not significantly different at the 5% probability level using LSD.

Table 6
Effect of CO_2 enrichment and light enhancement on photosynthate partitioning and acetylene reduction activity in Williams 82 (W82) and the NOD1-3 hypernodulating mutant

Cultivar	Light treatment	CO_2 treatment	Root/shoot ratio	(Total DW)/(nodule DW) ratio	ARA (μmol C_2H_4 plant^{-1} h^{-1})	Specific ARA (μmol C_2H_4 g^{-1} nodule DW h^{-1})
CO_2 effect						
W82	Low	Ambient	0.30[b]	24[a]	8.0[b]	112.1[a]
W82	Low	Enriched	0.36[a]	28[a]	15.1[a]	144.7[a]
NOD1-3	Low	Ambient	0.22[d]	8[b]	9.2[b]	65.3[b]
NOD1-3	Low	Enriched	0.25[c]	9[b]	14.0[a]	73.2[b]
Light effect						
W82	Low	Ambient	0.25[b]	28[a]	7.4[b]	87.2[a]
W82	High	Ambient	0.40[a]	27[a]	13.4[a]	95.4[a]
NOD1-3	Low	Ambient	0.21[c]	8[b]	6.9[b]	44.6[b]
NOD1-3	High	Ambient	0.24[b]	8[b]	11.2[a]	51.7[b]

Other plant growth and treatment conditions are as defined in the legend of Table 1. Within CO_2 or light effect, means with the same letter within columns are not significantly different at the 5% probability level using LSD.

alleviated by urea supplementation (Table 1), both light enhancement and CO_2 enrichment increased nodule number in both genotypes. This change of nodule number in Williams 82 with increased photosynthate supply under extended N supplementation (Table 1), which was lacking under limited N supplementation (Tables 2 and 3), may reflect the high sensitivity of nodulation in Williams 82 to excess N. In the presence of external N, the slower growth at low light or ambient CO_2 resulted in N accumulation and a feedback inhibition of nodulation [35] relative to faster growing plants (high light or CO_2 enrichment) where N was diluted. Light and CO_2 could have influenced change in nodule number through alteration in levels of the nodulation autoregulatory signal(s) or through alteration in photosynthate availability for root growth and nodulation without affecting levels of the autoregulatory signal. This latter possibility appears more likely as NOD1-3 remained hypernodulated and Williams 82 remained normally nodulated under all light and CO_2 levels. Also, under extended N supplementation, increased photosynthate supply increased nodule number in both the NOD1-3 hypernodulating mutant and the normal nodulating control. CO_2 enrichment increased plant growth and nodule number in the mutant more than in the control, while light enhancement increased plant growth and nodule number in the control more than in the mutant. Malik et al. [36] suggested that light may stimulate production of substances which can both inhibit infection and enhance development of established infections into nodules in soybean. However, reported non-photosynthetic effects of light on nodulation involve at least some aspect of light quality such as light versus darkness [36,37], far-red versus red light [38], or long versus short photoperiods [39] rather than light intensity alone. The red:far-red ratio in our low and high light levels (300 and 800 μmol m^{-2} s^{-1}, respectively) was similar, indicating that the observed light effect was purely photosynthetic.

Response of nodulation to photosynthate supply was positive under either light enhancement or CO_2 enrichment, but beyond this optimum, such as the increased light at full-strength nutrient solution, or high light × enriched CO_2 combination, or continuous light (24 h photoperiod), nodule number then decreased (Tables 1–3 and 5). This is consistent with the findings of Hansen et al. [11] in Bragg and its supernodulating mutants. They found that the maximum nodule number was obtained at intermediate light intensity (650

μmol m^{-2} s^{-1}) and nodule number was decreased at the maximum light intensity (1400 μmol m^{-2} s^{-1}). Thus, the nodule number response to photosynthate availability appears to reach a finite optimum, while increase in nodule mass continues with each increment increase in photosynthate supply.

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